

Supporting Information

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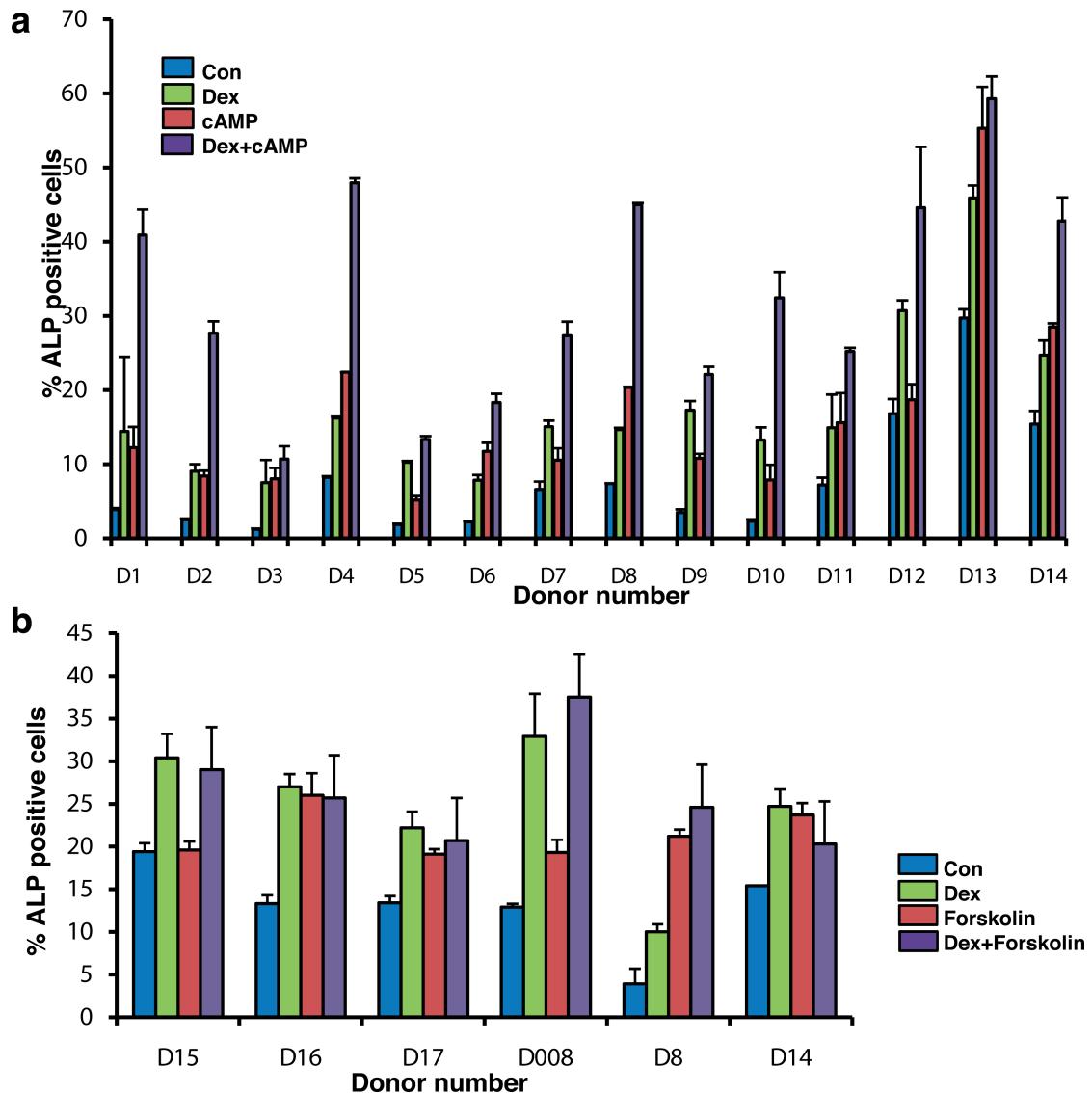


Fig. S1. (a) Percentage ALP-positive cells in hMSCs grown in basic medium (Con), osteogenic medium (Dex), basic medium supplemented with 1 mM db-cAMP (cAMP), or osteogenic medium supplemented with 1 mM db-cAMP (Dex+cAMP). (b) Percentage ALP-positive cells grown in basic medium (Con), osteogenic medium (Dex), basic medium supplemented with forskolin (Forskolin), or osteogenic medium supplemented with forskolin (Dex+Forskolin).

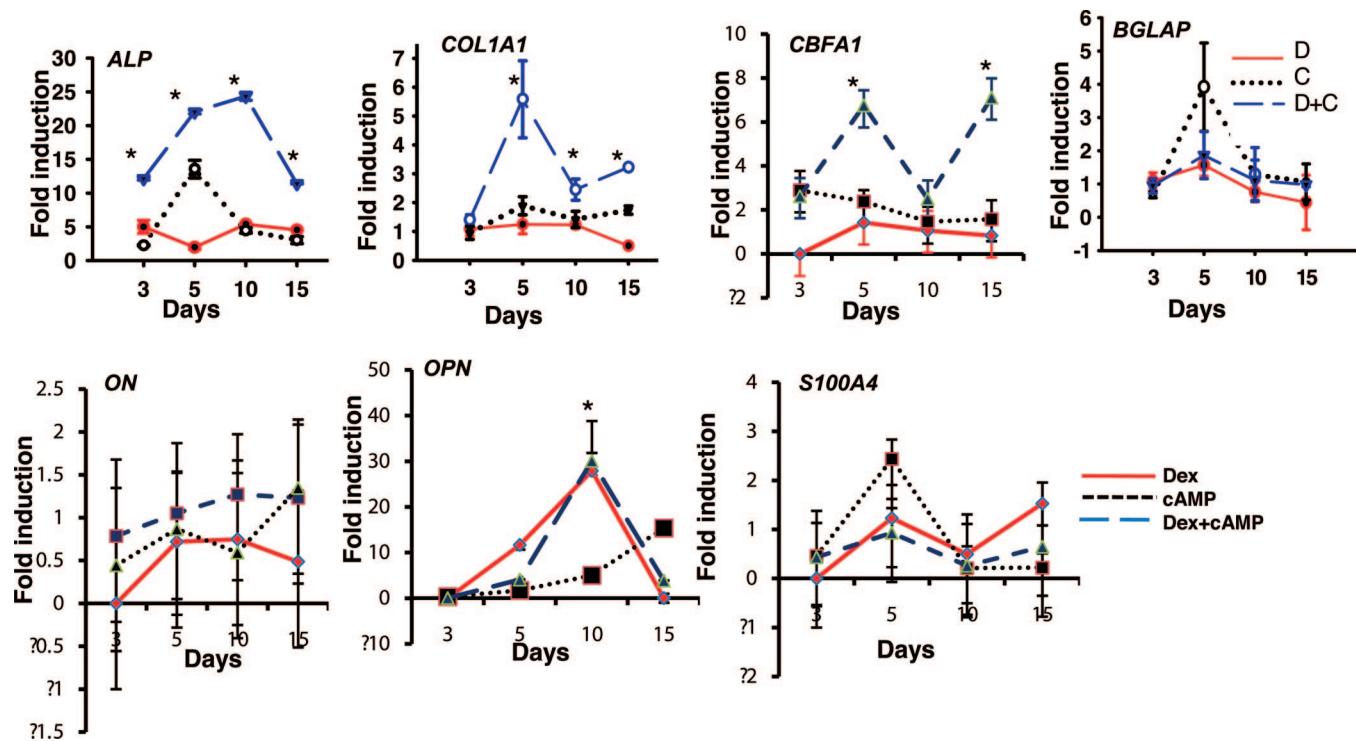


Fig. S2. hMSCs were grown in basic medium, basic medium supplemented with 1 mM db-cAMP (cAMP), osteogenic medium (Dex), or osteogenic medium supplemented with 1 mM db-cAMP (Dex+cAMP). Expression was analyzed by qPCR and is expressed as fold induction compared with cells grown in basic medium. The data were analyzed by using two-way ANOVA, and statistical significance is indicated compared with cells grown in basic medium. *, $P < 0.05$.

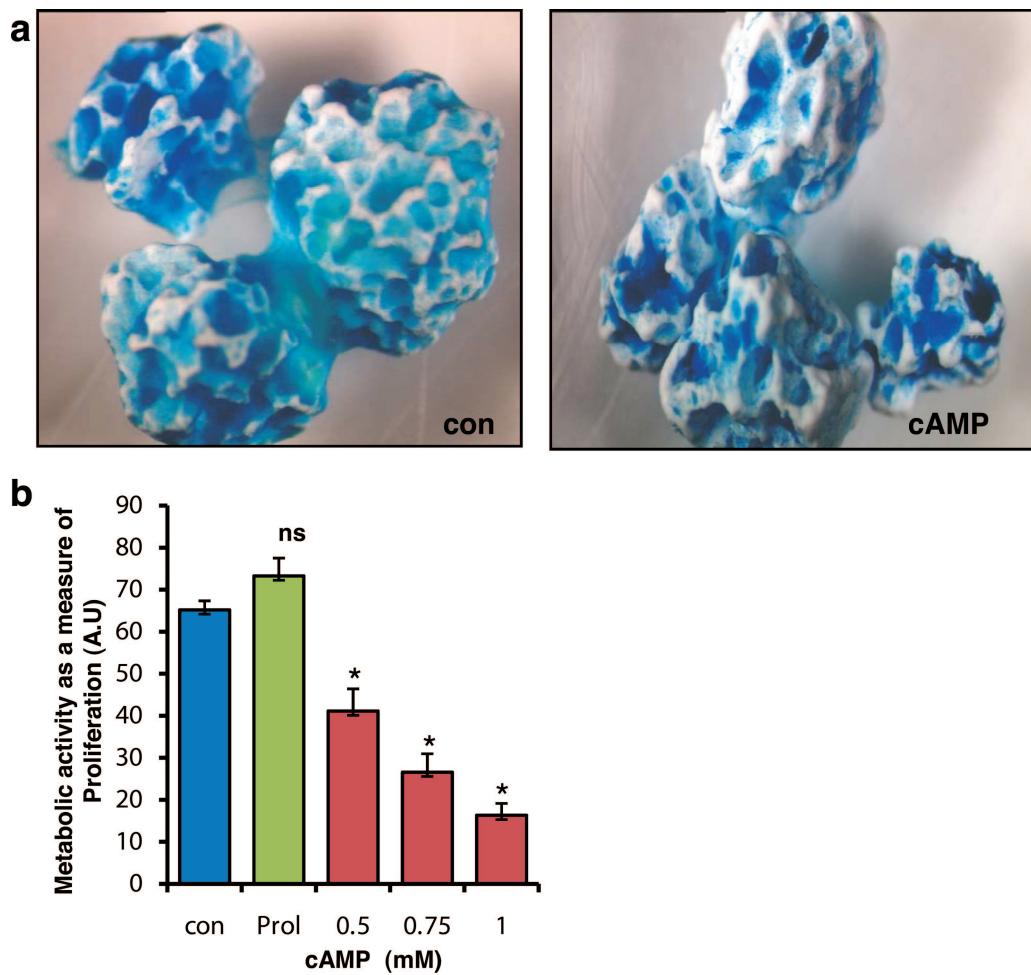


Fig. S3. (a) Methylene blue staining of hMSC-seeded scaffolds grown in basic medium (Con) or basic medium supplemented with 1 mM db-cAMP (cAMP) for 4 days. Note the less intensely stained db-cAMP-treated construct, indicating reduced cell numbers. (b) Quantitative Alamar blue assay for cell number analysis. The data were analyzed by using one-way ANOVA followed by Dunnett's multiple-comparison test. Statistical significance is indicated compared with cells grown in basic medium (Con). *, $P < 0.05$

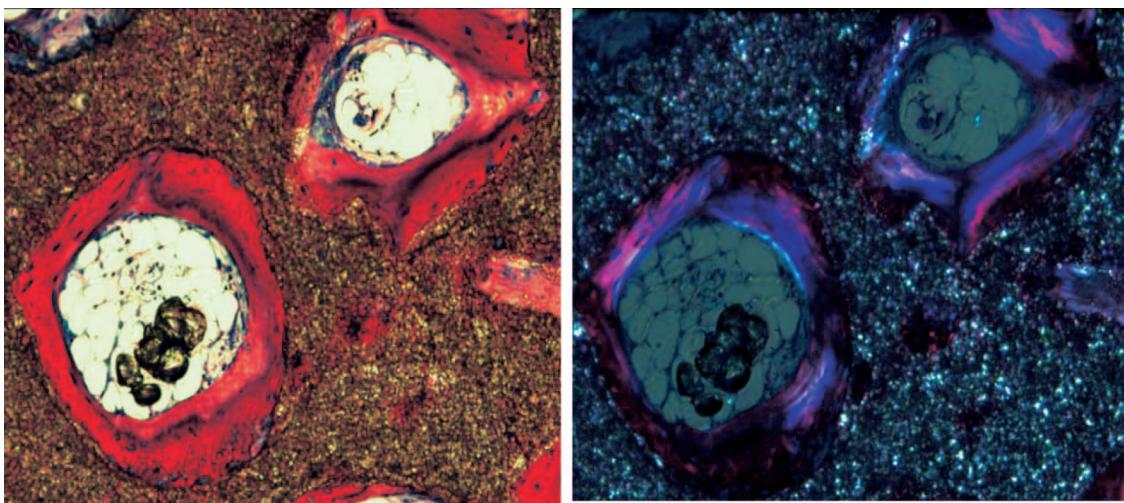


Fig. S4. A light microscopic image (*Left*) and polarized light microscopic image (*Right*) showing areas of polarized light indicating the presence of lamellar bone that has been remodeled by osteoclasts and osteoblasts.

Table S1. Donor information of hMSCs used in the study

Donor no.	Age, years	Sex	Source
D1	48	F	Iliac crest
D2	NA	NA	NA
D3	39	F	Iliac crest
D4	40	M	Iliac crest
D5	NA	NA	NA
D6	55	M	Iliac crest
D7	65	F	Acetabulum
D8	52	F	Acetabulum
D9	82	F	Acetabulum
D10	31	F	Acetabulum
D11	44	F	Acetabulum
D12	74	M	Acetabulum
D13	52	M	Acetabulum
D14	65	M	Acetabulum
D15	26	F	Iliac crest
D16	80	M	Acetabulum
D17	58	F	Acetabulum
D18	62	F	Acetabulum
D19	40	F	Acetabulum
D20	29	F	Iliac crest
D21	40	F	Iliac crest
D22	26	F	Iliac crest
D23	23	M	Iliac crest
D24	70	F	Iliac crest
D25	32	F	Iliac crest
D26	63	F	Iliac crest

F, female; M, male.

Table S2. Genes regulated >6-fold in 6-h db-cAMP-treated hMSCs

Accession no.	Sequence description	Fold change
NM_003979	retinoic acid induced 3	100.0
NM_031209	tRNA-guanine transglycosylase	25.3
AI972496	insulin-like growth factor 1 (somatomedin C)	19.1
NM_002048	growth arrest-specific 1	16.9
X75296	HIR (histone cell cycle regulation defective)	16.7
M57765	interleukin 11	15.4
NM_020634	growth differentiation factor 3	14.1
BC004490	v-fos FBJ murine osteosarcoma viral oncogene homolog	13.8
NM_002522	neuronal pentraxin I	12.8
BC002671	dual specificity phosphatase 4	12.1
AK000667	a disintegrin and metalloproteinase domain 15 (metarginidin)	12.0
NM_022453	ring finger protein 25	11.7
NM_005178	B-cell CLL/lymphoma 3	11.4
NM_001989	even-skipped homeo box 1 (homolog of Drosophila)	11.2
NM_002193	inhibin, β B (activin AB β polypeptide)	10.8
AW157094	inhibitor of DNA binding 4,	10.5
AL050152	neuronal specific transcription factor DAT1	10.3
D83485	glucose regulated protein, 58kD	10.3
NM_013281	fibronectin leucine rich transmembrane protein 3	9.4
NM_000579	chemokine (C-C motif) receptor 5	9.4
NM_006732	FBJ murine osteosarcoma viral oncogene homolog B	8.8
AL354872	Human DNA sequence from clone RP11-42O15 on chromosome 1.	8.7
NM_002579	paralemmin	8.4
NM_000529	melanocortin 2 receptor (adrenocorticotropic hormone)	7.8
NM_004983	potassium inwardly-rectifying channel, subfamily J, member 9	7.7
N26005	protein phosphatase 1, regulatory (inhibitor) subunit 3C	7.7
NM_001633	α -1-microglobulin/bikunin precursor	7.7
L24553	nitric oxide synthase 2A (inducible, hepatocytes)	7.7
NM_003534	H3 histone family, member H	7.6
NM_000560	CD53 antigen	7.5
NM_019598	Homo sapiens kallikrein 12 (KLK12), mRNA	7.5
U87964	GTP binding protein 1	7.5
NM_000197	hydroxysteroid (17- β) dehydrogenase 3	7.5
NM_015725	retinol dehydrogenase 8 (all-trans)	7.5
NM_002166	inhibitor of DNA binding 2,	7.4
NM_001523	hyaluronan synthase 1	7.3
NM_003085	synuclein, δ	7.3
NM_020639	ankyrin repeat domain 3	7.1
AB023167	lifeguard	7.0
NM_001718	bone morphogenetic protein 6	7.0
NM_016412	insulin-like growth factor 2, antisense	6.9
NM_001501	gonadotropin-releasing hormone 2	6.9
NM_012447	stromal antigen 3	6.9
NM_000552	von Willebrand factor	6.8
AL049250	Homo sapiens mRNA; cDNA DKFZp564D113	6.7
S62137	dopamine receptor D2	6.7
U51096	caudal type homeo box transcription factor 2	6.6
NM_003294	tryptase β 1	6.6
NM_002379	matrilin 1, cartilage matrix protein	6.6
NM_016321	Rh type C glycoprotein	6.6
NM_001269	chromosome condensation 1	6.5
AB018009	solute carrier family 7 (cationic amino acid transporter, y + system)	6.5
NM_016190	chromosome 1 open reading frame 10	6.5
NM_007185	trinucleotide repeat containing 4	6.4
NM_001886	crystallin, β A4	6.4
NM_002251	potassium voltage-gated channel, delayed-rectifier, subfamily S	6.4
NM_012146	double homeobox, 1	6.3
NM_003975	SH2 domain protein 2A	6.3
BC000019	cadherin 6, type 2, K-cadherin (fetal kidney)	6.3
NM_004693	cytokeratin type II	6.2
NM_001881	cAMP responsive element modulator	6.2

Table S3. Genes regulated >5-fold in 7-day db-cAMP-treated hMSCs

Accession no.	Sequence description	Fold change
NM_002993	small inducible cytokine subfamily B (Cys-X-Cys), GRO1 oncogene	60.6
NM_001511	interleukin 8	52.4
AF043337	bone morphogenetic protein 2	47.5
AA583044	secreted frizzled-related protein 4	46.4
AW089415	regulator of G-protein signalling 2, 24kD	37.1
NM_002923	frizzled (Drosophila) homolog 4	36.9
NM_012193	4-hydroxyphenylpyruvate dioxygenase	35.5
NM_002150	dual specificity phosphatase 4	32.1
BC002671	GRO3 oncogene	31.5
NM_002090	retinol-binding protein 1, cellular	31.4
NM_002899	CCAAT/enhancer binding protein (C/EBP), α	31.3
NM_004364	GRO2 oncogene	30.6
M57731	secretogranin II (chromogranin C)	30.1
NM_003469	cytokine-like protein C17	29.6
NM_018659	prostaglandin F receptor (FP)	29.1
NM_000959	tumor necrosis factor receptor superfamily, member 21	29.1
BE568134	γ -glutamyltransferase-like activity 1	26.3
NM_004121	potassium channel, subfamily K, member 15 (TASK-5)	25.5
NM_022358	PR domain containing 1, with ZNF domain	25.2
AL022067	I factor (complement)	23.2
NM_000204	H4 histone family, member D	22.8
NM_003541	secreted frizzled-related protein 1	22.1
NM_003012	tRNA-guanine transglycosylase	21.9
NM_031209	HIR (histone cell cycle regulation defective)	21.0
X75296	sialyltransferase 8 (α -2, 8-polysialytransferase) D	20.5
NM_005668	BTG family, member 2	19.9
NM_006763	interleukin 11	19.5
M57765	WNT1 inducible signaling pathway protein 2	19.2
NM_003881	a disintegrin-like and metalloprotease	18.9
NM_007038	insulin-like growth factor 1 (somatomedin C)	18.5
AI972496	serine/threonine kinase 16	18.2
AB020739	phosphatidic acid phosphatase type 2C	18.1
AF047760	heme oxygenase (decycling) 1	18.1
NM_002133	U2(RNU2) small nuclear RNA auxillary factor 1	18.0
NM_006758	cytokeratin type II	16.7
NM_004693	cathepsin C	16.6
NM_001814	a disintegrin and metalloproteinase domain 15 (metarginin)	16.3
AK000667	trinucleotide repeat containing 1	16.1
AI264312	CMRF35 leukocyte immunoglobulin-like receptor	15.7
NM_006678	retinal degeneration B β	15.7
NM_012417	bradykinin receptor B2	15.4
NM_000623	potassium voltage-gated channel, subfamily G, member 1	14.7
AI332979	apolipoprotein E	14.6
NM_000041	lung type-I cell membrane-associated glycoprotein	14.5
AU154455	HP1-BP74	14.1
NM_016287	platelet-derived growth factor receptor-like	14.0
NM_006207	synaptosomal-associated protein, 25kD	13.8
NM_003081	brain-specific membrane-anchored protein	13.4
NM_012109	hyaluronan synthase 1	13.2
NM_001523	G protein-coupled receptor, family C, group 5, member C	13.0
NM_022036	receptor tyrosine kinase-like orphan receptor 2	12.7
NM_004560	protein tyrosine phosphatase, receptor type, N	12.1
NM_002846	angiopoietin-like 2	12.1
NM_012098	phosphoinositide-3-kinase, regulatory subunit, (p55, γ)	12.0
BE622627	RAB-8b protein	11.7
NM_016530	leucine-rich and death domain containing	11.6
NM_018494	pleckstrin homology-like domain, family A, member 1	11.6
AA576961	glucose regulated protein, 58kD	11.3
D83485	cytochrome P450, subfamily XXVIIA	11.2
NM_000784	suppressor of var1 (<i>S.cerevisiae</i>) 3-like 1	11.2
NM_003171	ring finger protein 25	11.2
NM_022453	spinal cord-derived growth factor-B	10.4
NM_025208	aldehyde dehydrogenase 1 family, member A3	10.2

Accession no.	Sequence description	Fold change
NM_012254	very long-chain acyl-CoA synthetase homolog 2	10.2
AV753028	transducin (β)-like 1	9.9
AF097419	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor-like 1	9.8
NM_005025	serine (cysteine) proteinase inhibitor, clade I (neuroserpin)	9.8
NM_013261	PPR- γ , coactivator 1	9.7
AF260261	Homo sapiens Abl-interactor protein 2b (ABI2B) mRNA,	9.6
NM_004398	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 10	9.6
NM_001248	ectonucleoside triphosphate diphosphohydrolase 3	9.5
NM_001998	fibulin 2	9.5
NM_005410	selenoprotein P, plasma, 1	9.4
AB029343	HCR (a-helix coiled-coil rod homologue) gene, complete cds.	9.3
NM_007115	tumor necrosis factor, α -induced protein 6	9.2
AB006757	BH-protocadherin (brain-heart)	9.2
AF012074	phosphodiesterase 4D, cAMP-specific	9.0
NM_001517	general transcription factor IIH, polypeptide 4	9.0
NM_001804	caudal type homeo box transcription factor 1	9.0
NM_018096	hypothetical protein similar to β -transducin family	8.9
U12707	Wiskott-Aldrich syndrome (eczema-thrombocytopenia)	8.9
NM_006614	cell adhesion molecule with homology to L1CAM	8.9
BC000563	solute carrier family 6 (neurotransmitter transporter, noradrenalin), member 2	8.7
NM_002585	pre-B-cell leukemia transcription factor 1	8.7
NM_007185	trinucleotide repeat containing 4	8.6
NM_001415	eukaryotic translation initiation factor 2, subunit 3 (γ)	8.6
AK021882	ras homolog gene family, member I	8.6
NM_018398	calcium channel, voltage-dependent, α 2/delta 3 subunit	8.6
NM_005646	TAR (HIV) RNA-binding protein 1	8.4
NM_001519	TATA box binding protein (TBP)-associated factor,	8.4
NM_002231	kangai 1	8.3
NM_002674	pro-melanin-concentrating hormone	8.2
NM_001670	armadillo repeat gene deletes in velocardiofacial syndrome	8.2
NM_021800	J domain containing protein 1	8.2
AB009598	β -1,3-glucuronidyltransferase 3 (glucuronosyltransferase I)	8.1
NM_004285	hexose-6-phosphate dehydrogenase (glucose 1-dehydrogenase)	8.1
L20966	phosphodiesterase 4B, cAMP-specific	8.0
NM_002120	major histocompatibility complex, class II, DO β	8.0
NM_005213	cystatin A (stefin A)	7.9
NM_000809	γ -aminobutyric acid (GABA) A receptor, α 4	7.9
NM_004566	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3	7.9
NM_014343	claudin 15	7.9
NM_005771	retinol dehydrogenase homolog	7.9
L12711	transketolase (Wernicke-Korsakoff syndrome)	7.9
NM_015714	putative lymphocyte G0/G1 switch gene	7.9
NM_005019	phosphodiesterase 1A, calmodulin-dependent	7.8
NM_018933	protocadherin β 13	7.7
NM_004961	γ -aminobutyric acid (GABA) A receptor, epsilon	7.6
BF575514	pre-B-cell colony-enhancing factor	7.5
NM_003427	zinc finger protein 76 (expressed in testis)	7.5
BC000658	stanniocalcin 2	7.4
AF284095	adrenergic, α -2A-, receptor	7.4
NM_016585	testicular haploid expressed gene	7.4
NM_002281	keratin, hair, basic, 1	7.4
NM_002610	pyruvate dehydrogenase kinase, isoenzyme 1	7.3
NM_005230	ELK3, ETS-domain protein (SRF accessory protein 2)	7.3
NM_022467	carbohydrate (N-acetylgalactosamine 4-O) sulfotransferase 8	7.3
NM_006227	phospholipid transfer protein	7.3
NM_020133	lysophosphatidic acid acyltransferase- δ	7.3
NM_006271	S100 calcium-binding protein A1	7.2
NM_002427	matrix metalloproteinase 13 (collagenase 3)	7.2
NM_013281	fibronectin leucine rich transmembrane protein 3	7.1
AF105974	hemoglobin, α 2	7.1
NM_000850	glutathione S-transferase M4	7.1
NM_014512	killer cell immunoglobulin-like receptor,	7.1
NM_000451	short stature homeobox	7.0
NM_004313	arrestin, β 2	7.0
NM_025263	CAT56 protein	7.0

Accession no.	Sequence description	Fold change
NM_005261	GTP-binding protein overexpressed in skeletal muscle	7.0
NM_006675	tetraspan transmembrane 4 super family	7.0
AI433463	membrane metallo-endopeptidase	6.9
M60334	major histocompatibility complex, class II, DR α	6.9
L76668	killer cell immunoglobulin-like receptor	6.8
AL554008	G protein-coupled receptor 56	6.8
NM_004387	cardiac-specific homeo box	6.8
AF133425	tetraspan 1	6.7
BC002538	serine (or cysteine) proteinase inhibitor, clade B (ovalbumin)	6.7
NM_004139	lipopolysaccharide-binding protein	6.6
NM_001993	coagulation factor III (thromboplastin, tissue factor)	6.6
AF216693	interleukin-1 receptor antagonist homolog 1 (IL1HY1)	6.6
NM_014434	NADPH-dependent FMN and FAD containing oxidoreductase	6.6
NM_000537	Rennin	6.6
NM_000898	monoamine oxidase B	6.6
J03225	tissue factor pathway inhibitor	6.6
NM_005227	ephrin-A4	6.5
AW084582	splicing factor, arginine/serine-rich 5	6.5
NM_005173	ATPase, Ca ⁺⁺ transporting, ubiquitous	6.5
NM_006332	interferon, γ -inducible protein 30	6.5
NM_004694	solute carrier family 16 (monocarboxylic acid transporters)	6.4
NM_005524	hairy (Drosophila)-homolog	6.4
NM_006078	calcium channel, voltage-dependent, γ subunit 2	6.4
NM_022661	SPANX family, member C	6.4
NM_003654	carbohydrate (keratan sulfate Gal-6) sulfotransferase 1	6.3
NM_000727	calcium channel, voltage-dependent, γ subunit 1	6.3
BC001422	placental growth factor, vascular endothelial growth factor-related protein	6.3
AB018009	solute carrier family 7	6.2
NM_001269	chromosome condensation 1	6.2
NM_004426	early development regulator 1 (homolog of polyhomeotic 1)	6.2
NM_004982	potassium inwardly-rectifying channel, subfamily J	6.2
NM_003531	H3 histone family, member C	6.1
AV724216	NDRG family, member 4	6.1
NM_022720	DiGeorge syndrome critical region gene 8	6.1
M65062	insulin-like growth factor binding protein 5	6.1
AF217487	killer cell immunoglobulin-like receptor, two domains, Ig	6.1
AB014719	amyloid β (A4) precursor protein-binding, family A,	6.1
U35004	mitogen-activated protein kinase 8	6.0
AL513917	solute carrier family 16 (monocarboxylic acid transporters)	6.0
NM_006250	proline-rich protein Haell subfamily 1	6.0
NM_007009	zona pellucida binding protein	6.0
NM_021046	UHS KerB	6.0
NM_005222	distal-less homeo box 6	5.9
NM_003116	sperm associated antigen 4	5.9
NM_021181	19A24 protein	5.8
NM_016582	peptide transporter 3	5.8
NM_019598	Homo sapiens kallikrein 12 (KLK12), mRNA	5.8
NM_000924	phosphodiesterase 1B, calmodulin-dependent	5.8
NM_015872	kruppel-related zinc finger protein hcKrox	5.8
NM_014571	hairy/enhancer-of-split related with YRPW motif-like	5.8
NM_022728	neurogenic differentiation 6	5.8
NM_018441	peroxisomal trans 2-enoyl CoA reductase	5.7
NM_030772	connexin 59	5.7
NM_004794	RAB33A, member RAS oncogene family	5.7
NM_004148	ninjurin 1	5.6
NM_005076	contactin 2 (axonal)	5.6
NM_006037	histone deacetylase 4	5.5
M69148	midkine (neurite growth-promoting factor 2)	5.5
NM_012351	olfactory receptor, family 10, subfamily J, member 1	5.5
BF061658	transforming growth factor, β 2	5.4
NM_018939	protocadherin β 6	5.4
NM_001406	ephrin-B3	5.4
NM_001958	eukaryotic translation elongation factor 1 α 2	5.4
NM_018937	protocadherin β 3	5.4
NM_030930	unc93 (C.elegans) homolog B	5.4

Accession no.	Sequence description	Fold change
U91903	frizzled-related protein	5.4
AF172331	regenerating islet-derived 1 α	5.4
NM_005266	gap junction protein, α 5, 40kD (connexin 40)	5.4
NM_000129	coagulation factor XIII, A1 polypeptide	5.4
NM_002479	myogenin (myogenic factor 4)	5.4
AA988241	RAB3A, member RAS oncogene family	5.3
NM_001169	aquaporin 8	5.3
NM_002573	platelet-activating factor acetylhydrolase, isoform Ib,	5.3
NM_018684	hepatocellular carcinoma-associated antigen 127	5.3
NM_003508	frizzled (Drosophila) homolog 9	5.3
NM_014580	solute carrier family 2, (facilitated glucose transporter)	5.3
AJ297586	major histocompatibility complex, class II, DR β 5	5.3
NM_022047	differentially expressed in FDCP (mouse homolog) 6	5.3
NM_003967	putative neurotransmitter receptor	5.3
NM_005029	paired-like homeodomain transcription factor 3	5.3
NM_002339	lymphocyte-specific protein 1	5.2
NM_006419	small inducible cytokine B subfamily	5.2
U65590	Homo sapiens IL-1 receptor antagonist IL-1Ra	5.2
NM_016109	angiotropin-like 4	5.2
NM_020547	anti-Mullerian hormone receptor, type II	5.2
NM_005130	heparin-binding growth factor binding protein	5.2
NM_014440	interleukin 1, epsilon	5.1
NM_000458	transcription factor 2, hepatic; LF-B3;	5.1
BC003143	dual specificity phosphatase 6	5.1
NM_006695	Rap2 interacting protein 8	5.1
AF031924	BarH-like homeobox 2	5.0
NM_002036	Duffy blood group	5.0
NM_021158	protein kinase domains containing protein similar to phosphoprotein C8FW	5.0
NM_014298	quinolinate phosphoribosyltransferase	5.0